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Evidence of Introgressive Hybridization between Bull Trout and Brook Trout

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Abstract.—Hybridization between native bull trout *Salvelinus confluentus* and introduced brook trout *S. fontinalis* occurs over a wide geographic area in the western United States. We described the extent to which introgressive hybridization has occurred between these species using biochemical and molecular genetic techniques in samples collected from five streams in western Montana. We found that about three-quarters of the hybrids detected were male, first-generation (F_1) hybrids. Most of the rest were backcrosses to the parental species, indicating that F_1 hybrids can reproduce. We found no evidence of hybrid swarms in which all individuals were of hybrid origin. Our results suggest that both the reduced fertility of F_1 hybrids and the reduced survival of their progeny prevents these species from forming such hybrid swarms. We also found that hybridization between bull and brook trout tends to occur predominantly between female bull trout and male brook trout, indicating that hybridization represents greater wasted reproductive effort for bull trout than for brook trout.

Bull trout *Salvelinus confluentus* are now legally protected as threatened in the United States under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service 1999). Hybridization with introduced brook trout *S. fontinalis* is potentially one of the major threats to the persistence of bull trout (Markle 1992; Leary et al. 1993, 1995; Buktenica 1997). Bull trout and brook trout have no overlap in their natural distribution (Meehan and Bjornn 1991), but secondary contact between these species has occurred as a result of the introduction of brook trout into the bull trout's native range (Evermann 1901; MacCrimmon and Campbell 1969).

Leary et al. (1993) described a rapid and almost complete displacement of bull trout by brook trout in which the initial phases were characterized by frequent hybridization. In the South Fork of Lolo Creek in the Bitterroot River drainage, Montana, brook trout first invaded in the late 1970s. In the initial sample collected in 1982, bull trout (43.6%) were the most abundant, followed by hybrids (35.9%) and brook trout (20.5%), and matings appeared to be occurring at random. By 1990, however, brook trout (64.7%) were more abundant than bull trout (23.5%) and hybrids (11.8%). The authors suggested that hybridization might aid the displacement of bull trout because reproductive effort is wasted in hybrid production. Brook trout may also displace bull trout by competition because of their short life cycle, wider habitat preference, and tendency to overpopulate small streams (Scott and Crossman 1973).

A number of first-generation (F_1) hybrids between bull and brook trout have been detected (Leary et al. 1983, 1993, 1995). Using protein electrophoresis, Leary et al. (1983) identified hybrids between these species in samples collected from three streams in western Montana and found that all hybrids ($N = 20$) from these streams were F_1 males. Furthermore, Leary et al. (1995) reported that 50 of 53 hybrid fish collected from nine tributaries to the Bitterroot River, Montana, were identified as F_1 on the basis of protein electrophoresis (see also Buktenica 1997). Two of the remaining hybrids appeared to be backcrosses to bull trout, and the third appeared to be a backcross to brook trout; this suggested that there was some isolating mechanism that prevented the species from forming hybrid swarms in which essentially all fish are of hybrid origin.

The near absence of progeny from hybrids of bull and brook trout in these streams may result from either the sterility of the hybrids, their lack of mating success, the poor survival of their progeny, or combinations of these factors. The largely complete sterility of the F_1 hybrids has been suspected from the finding that those collected from several places in northwestern United States were exclusively male (Leary et al. 1983, 1993, 1995).

Although extensive introgressive hybridization between bull and brook trout has not been detected, there is no doubt that hybrids beyond F_1

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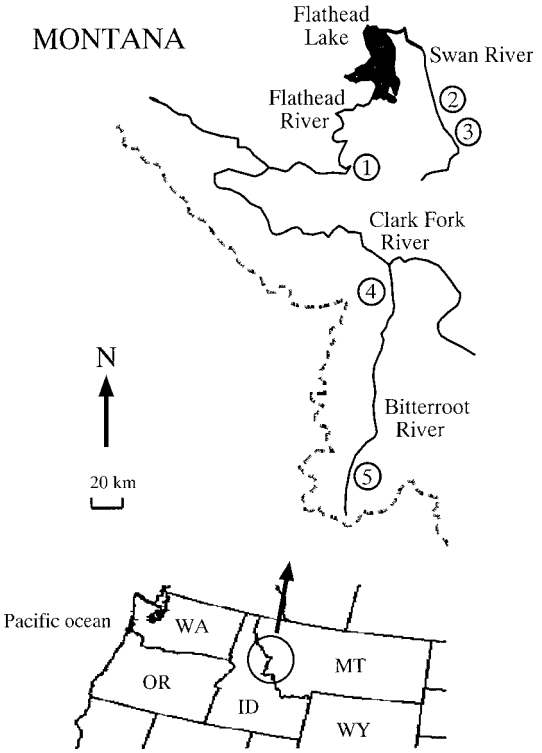

FIGURE 1.—Sampling locations in western Montana: 1 = Mission Creek, 2 = Goat Creek, 3 = Lion Creek, 4 = One Horse Creek, and 5 = Slate Creek.

exist in the wild. Conservation management practices that ignore the fact that F_1 hybrids can reproduce could result in the delay of bull trout recovery or the local extinction of bull trout since backcrossing also represents wasted reproductive effort. The primary objective of this paper was to describe the extent of introgressive hybridization between bull trout and brook trout using both biochemical and molecular genetic techniques. Genetic markers can identify a hybrid as an F_1, a backcross, or later-than-first-generation hybrid with high confidence. In contrast to previous studies, we detected a surprisingly high number of backcrosses and F_2 hybrids. We also tested the possible sterility of hybrids by determining their sex. These results allowed us to investigate whether or not the reduced fertility of F_1 hybrids and the lower survival of their progeny are responsible for the lack of hybrid swarms. Finally, we determined the direction of hybridization by analyzing mitochondrial DNA extracted from F_1 hybrids in order to investigate the relative amount of reproductive effort wasted by bull trout females in the production of hybrids.

Methods

Samples.—Samples were collected by electrofishing and were sent to our laboratory in several forms: the whole body, liver, eye, and muscle tissues, or fin clips. Fin clips were sent either frozen or stored in a 95% solution of ethanol; those in ethanol were not used for allozyme analysis. The sample locations, all in Montana, were as follows: upper Mission Creek in the lower Flathead River drainage; Lion and Goat creeks in the Swan River drainage; Slate Creek in the West Fork of the Bitterroot River drainage; and One Horse Creek in the northwestern Bitterroot River drainage (Figure 1; Table 1). These sample locations were selected because previous results indicated that the streams contained hybrids (Leary et al. 1993; R. F. Leary, unpublished data).

The life histories of bull and brook trout differ among the creeks. In Slate and One Horse creeks both species are mainly, if not exclusively, resident fish that spend their entire lives in the streams. In Mission Creek both species include migratory fish that mature in Mission Reservoir and spawn in the creek (Hansen and DosSantos 1997); Mission Creek also contains resident brook trout but apparently not resident bull trout. The bull trout in Lion and Goat creeks are migratory, maturing in Swan Lake and spawning in the creeks (Montana Bull Trout Scientific Group 1996); the brook trout in those creeks are resident.

All the samples collected from Mission Creek in 1993–1995 and some (N = 24) of the samples collected in 1996 contained suspected hybrid fish

TABLE 1.—Sampling locations and the number of fish collected. Location numbers correspond to those in Figure 1.

Location (drainage)	Sampling year				
	1992	1993	1994	1995	1996
1. Mission Creek (Flathead River)		16	37	23	88
2. Goat Creek (Swan River)	15	15			
3. Lion Creek (Swan River)		45			
4. One Horse Creek (Bitterroot River)					35
5. Slate Creek (Bitterroot River)				21	40

that were selectively kept for analysis while suspected parentals were released. The samples from Lion and Goat creeks were also not randomly collected because all fish were initially considered to be bull trout (Kanda et al. 1997). Therefore, meaningful estimation of the proportion of hybrids in most of the samples was not possible. The only random samples in which we kept all the fish collected (as either whole bodies or fin clips) were from Mission Creek in 1996 ($N = 64$), Slate Creek, and One Horse Creek.

Protein electrophoresis.—Horizontal starch gel electrophoresis followed the procedures of Leary and Booke (1990). The products of eight loci coding for enzymes present in the muscle or liver that are known to be diagnostic between bull and brook trout were analyzed (Leary et al. 1983, 1993): aspartate aminotransferase (2.6.1.1 [IUBNC 1984]; *sAAT-1**), creatine kinase (2.7.3.2; *CK-A1**), L-iditol dehydrogenase (1.1.1.14; *IDDH**), isocitrate dehydrogenase (1.1.1.42; *sIDHP-2**), L-lactate dehydrogenase (1.1.1.27; *LDH-A1**, *LDH-B2**), malate dehydrogenase (1.1.1.37; *sMDH-A2**), and superoxide dismutase (1.15.1.1; *sSOD-1**). The tissues from which the enzymes were obtained and the electrophoretic buffers used for their analysis are discussed in Leary et al. (1993) and Kanda et al. (1997). The stains used to reveal the position of particular enzymes in the gels after electrophoresis followed the recipes of Harris and Hopkinson (1976) and Allendorf et al. (1977). The nomenclature for loci and alleles follows the recommendations of Shaklee et al. (1990). Allelic mobilities are relative to the product of the common allele at each homologous locus in Arlee rainbow trout *Oncorhynchus mykiss* maintained by the Montana Department of Fish, Wildlife, and Parks at the Jocko River State Trout Hatchery, Arlee.

DNA isolation.—Genomic DNA was isolated from either frozen muscle or fin clips using the Puregene DNA isolation kit (Gentra System). The extracted DNA concentration was determined using agarose gel electrophoresis, diluted when necessary to the appropriate concentrations for polymerase chain reaction (PCR) amplification.

Nuclear DNA markers.—Different combinations of two nuclear DNA primers were used to produce diagnostic fragments between bull and brook trout in acrylamide gels. We call this PCR-based method PINE-PCR because it utilizes paired interspersed nuclear DNA elements (Spruell et al. 2001). The sequences of PCR primers were made complementary to the end of consensus sequences of short interspersed nuclear DNA element families (SINE; Kido et al. 1991), namely, *Hpa*I (5′-AACCACT AGGCTACCCTGCC-3′), *Fok*I (5′-CCAACTGA GCCACACGGGAC-3′), and *Sma*I (5′-AACTGA GCTACAGAAGGACC-3′). These SINE families are known to be ubiquitous in the genomes of the genus *Salvelinus*, and the PCR amplifies the intergenomic fragments (i.e., PINE fragments) between the ends of the SINE elements. Gel images of the diagnostic PINE fragments are provided elsewhere (Spruell et al. 2001; Kanda et al. 2002). Although data demonstrating the Mendelian inheritance of the diagnostic PINE fragments are not available, such polymorphisms are known to be heritable in Mendelian fashion in pink salmon *O. gorbuscha* (Spruell et al. 1999) and undoubtedly are in *Salvelinus*.

PINE fragments are scored as the presence or absence of a particular fragment of a particular base pair (bp) length. Fixed differences in the presence or absence of the fragments between different species are used to detect hybrids (Spruell et al. 2001). Six fragments (432, 290, 236, 225, 192, and 115 bp) from the *Hpa*I−*Fok*I combination and eight fragments (303, 293, 240, 226, 144, 130, 118, and 105 bp) from the *Hpa*I−*Sma*I combination are diagnostic between bull and brook trout (Spruell et al. 2001). Ten fragments are specific to bull trout: the 432-, 236-, 225-, and 115-bp *Hpa*I−*Fok*I fragments and the 303-, 240-, 226-, 144-, 118-, and 105-bp *Hpa*I−*Sma*I fragments. Four fragments are specific to brook trout: the 290- and 192-bp *Hpa*I−*Fok*I fragments and the 293- and 130-bp *Hpa*I−*Sma*I fragments.

PCR amplification was performed in a 10-μL reaction solution containing 100 ng of DNA, 10 pmol of each primer, 0.4 unit of *Taq* polymerase (Perkin-Elmer), 1 μL each of 2mM deoxynucleotide triphosphate mix, 1 μL of 10× reaction buffer (Perkin-Elmer), and 1.5 μL of 25mM $MgCl_2$. The PCR profile consisted of denaturation at 93°C for 3 min followed by 30 cycles of 1 min of denaturation at 92°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C. Amplified products were run in 7% acrylamide gels and visualized using fluorescent images with a Hitachi FMBIO100 imager.

Probability of hybrid identification.—Allozymes are codominant markers, as alleles characteristic of both parental species are readily detectable in heterozygous individuals (e.g., Leary et al. 1983). The F_1 hybrids were determined to be the fish that were heterozygous for alleles characteristic of both bull and brook trout at all the diagnostic loci. Backcross fish, those produced from crosses be-

TABLE 2.—The number of individuals genetically determined to be bull trout, brook trout, F_1 hybrids, backcrosses to bull trout (BBL), backcrosses to brook trout (BBR), and F_2 hybrids using allozymes and paired interspersed nuclear DNA elements (PINEs). Liver and muscle allozymes provided eight diagnostic loci, fin allozymes three diagnostic loci; PINEs provided 10 markers specific to bull trout and 4 specific to brook trout.

Marker	Genetic status of fish						Total
	Bull trout	Brook trout	F_1 hybrids	BBL	BBR	F_2 hybrids	
Allozymes							
Liver and muscle	69	96	59	5	17	2	248
Fin	14	4	17	0	0	1	36
PINEs	60	3	31	4	8	2	108
Allozymes and PINEs							
Liver and muscle	0	2	17	1	6	3	29
Fin	12	1	11	2	0	2	28

tween F_1 hybrids and one of the parental species, were identified as fish that were heterozygous at some diagnostic loci and homozygous for alleles characteristic of only one of the species at other diagnostic loci. F_2 or later-generation hybrids, those produced from crosses between F_1 hybrids, were identified as fish that were heterozygous at some diagnostic loci and homozygous for alleles characteristic of both parental species at other diagnostic loci.

The presence of a PINE fragment is dominant to absence because a heterozygote possesses the fragment. The appearance of a fragment, therefore, indicates that the individual is either heterozygous or homozygous for the fragment. The F_1 hybrids were thus determined to be those fish that had all 14 of the diagnostic fragments analyzed between the two species. Backcrosses to bull trout were fish that had all bull trout diagnostic fragments and one to three brook trout diagnostic fragments. Backcrosses to brook trout were fish that had all brook trout diagnostic fragments and one to nine bull trout diagnostic fragments. The F_2 or later-generation hybrids were fish that had one to nine bull trout fragments and one to three brook trout fragments.

Using the above criteria, we calculated the probabilities of misidentifying individual fish using allozymes, PINEs, or both by means of the equations in Appendix 1. With allozymes, the probabilities of misidentifying individual fish with the eight diagnostic loci are quite low, except that there is an approximately 20% chance of misclassifying an F_2 hybrid as a backcross. For the purpose of examining the extent of introgressive hybridization, therefore, our genetic identification of fish using allozymes from muscle and liver tissue is very reliable. Only the extent of backcross matings is likely to be overestimated to the same extent that matings between F_1 hybrids are underestimated. The probabilities of misidentification, however, generally increase by more than one order of magnitude when we analyze allozymes from fin clips for hybrid identification, since only three of the eight diagnostic loci can be used. In fact, there is a better than 50% chance that an F_2 individual will be misclassified as a backcross.

With PINEs, there is a good chance (30%) of misidentifying an F_2 individual as a backcross to brook trout and a fair chance (6.3%) of misidentifying a backcross to bull trout as a bull trout or an F_1 hybrid. Thus, the use of PINEs will tend to slightly overestimate the number of bull trout, F_1 hybrids, and backcrosses to brook trout and to underestimate the number of F_2 hybrids and backcrosses to bull trout.

Using both allozymes and PINEs, the probabilities of misidentifying individual fish are very low, except that there is an approximately 10% chance of misclassifying an F_2 hybrid as a backcross when only fin clips are available. The combined use of allozymes and PINEs is the most reliable method for hybrid detection because it employs the greatest number of diagnostic loci.

Most individuals were identified from whole-body samples using eight diagnostic allozyme loci or from fin clip samples using PINEs (Table 2). There is a reasonable chance that a single backcross individual in our samples was misidentified as a parental or F_1. There is also a reasonable chance that an F_2 individual was misidentified as an F_1 or backcross when only allozymes or PINEs were used. Thus, it is likely that at worst only two individuals in our data set were misidentified. Such errors should have little influence on our conclusions, as the vast majority of fish were correctly identified.

Mitochondrial DNA.—We used restriction fragment length polymorphism of the NADH dehy-

TABLE 3.—The number of F_1 hybrids between bull and brook trout, backcrosses, and F_2 hybrids detected; mtDNA haplotypes of F_1 hybrids; and sexes of F_1 hybrids, backcrosses, and F_2 hybrids. Ten F_1 hybrids from Mission Creek were not analyzed for mtDNA haplotype because they were discarded before DNA was extracted; M = male, F = female.

		F_1 hybrids					Backcross			F_2 hybrids		
			mtDNA		Sex			Sex			Sex	
Stream	Number of hybrids	*N*	Bull	Brook	M	F	*N*	M	F	*N*	M	F
Mission Creek	71	51	22	19	6	3	15	7	1	5	2	0
Goat Creek	1	0	0	0	0	0	1	0	0	0	0	0
Lion Creek	13	12	12	0	12	0	1	0	0	0	0	0
One Horse Creek	12	9	9	0	9	0	3	2	0	0	0	0
Slate Creek	10	5	5	0	5	0	5	2	3	0	0	0
Total	107	77	48	19	32	3	25	11	4	5	2	0

drogenase 5 and 6 (ND5/6) region of mitochondrial DNA (mtDNA) to examine the direction of parental matings that produced F_1 hybrids. Primer sequences for the ND5/6 region are described by Cronin et al. (1993).

PCR amplifications were performed in 20-μL reaction mixtures containing 100 ng of DNA, 8 pmol of each primer, 0.5 unit of *Taq* polymerase (Perkin-Elmer), and 2 μL of 2mM deoxynucleotide triphosphate mix, 2 μL of 25mM $MgCl_2$, and 2 μL of 10× reaction buffer (Perkin-Elmer). The PCR profile consisted of denaturation at 95°C for 1 min followed by 30 cycles of 1 min of denaturation at 92°C, 1 min of annealing at 50°C, and 1.5 min of extension at 72°C.

Amplified segments were digested using *Cfo*I and *Rsa*I restriction enzymes. Restriction fragments of the ND5/6 region digested by these two enzymes showed fixed differences between bull and brook trout (Williams et al. 1997). Digests were performed in 10-μL volumes containing 3 μL of PCR product, 2–3 units of enzyme, and 1 μL of 10× digestion buffer. The digested fragments were separated by 2.5% agarose gel electrophoresis with ethidium bromide and visualized by UV transillumination or by a Hitachi FMBIO100 imager.

Sex of hybrid fish.—The sex of F_1 hybrids was determined either by examining their gonads under a binocular microscope or by unaided visual inspection, depending on size. Thus, we could have misidentified sterile females as immature or sterile males because their gonads sometimes look similar unless histochemically stained. The number of F_1 hybrids from Mission Creek for which the sex could be determined was limited to those nine sampled as whole fish.

Results

Genetic Identification

We detected hybrids from all the streams sampled (Table 3). More than 30% (107/335) of the fish analyzed were hybrids. Most of them (77) were F_1, but a considerable number of backcrosses and F_2 hybrids were also detected (hereafter, backcrosses and F_2 hybrids will be collectively called post-F_1 hybrids unless otherwise specified). We found that 20 of 71 hybrids from Mission Creek, 5 of 10 from Slate Creek, 3 of 12 from One Horse Creek, 1 of 13 from Lion Creek, and the only hybrid fish from Goat Creek were post-F_1 (Table 3). The highest number of the hybrids was detected in the Mission Creek sample because suspected hybrids were selectively kept for the genetic analysis. These results indicate that F_1 hybrids are capable of reproducing and that their reproduction is geographically widespread.

Twenty-five of the 30 post-F_1 hybrids were backcrosses to one of the parental species (Table 3). These fish were either (1) heterozygous at some diagnostic allozyme loci and homozygous for alleles characteristic of one of the parental species at the other diagnostic allozyme loci or (2) possessed all PINE markers characteristic of one of the parental species and lacked some, but not all, of the PINE markers characteristic of the other parental species. The genotypes at the allozyme diagnostic loci and the distribution of PINE markers indicated that 9 were backcrosses to bull trout and 16 were backcrosses to brook trout. The remaining 5 fish (all of which were collected from Mission Creek) were F_2 hybrids because they were homozygous for alleles characteristic of both parental taxa at some diagnostic allozyme loci and possessed some, but not all, of the PINE markers characteristic of both bull and brook trout.

Sex of Hybrid Fish

Overall, 32 of the 35 F_1 hybrids sexed appeared to be males (Table 3). Only the Mission Creek sample contained female F_1 hybrids, and these 3 fish were gravid with eggs. These female F_1 hybrids are unlikely to be backcrosses or misidentified F_2 hybrids because two of them were identified using both allozymes and PINEs and the other one using eight diagnostic allozyme loci. Two of the three female F_1 hybrids had brook trout mtDNA and the other one had bull trout mtDNA, indicating that both bull trout female × brook trout male and brook trout female × bull trout male matings can produce female F_1 hybrids. Of the 15 backcrosses and 2 F_2 hybrids whose sex could be determined, most were males, but 3 backcross females were collected from Slate Creek and 1 from Mission Creek (Table 3). Evidence of male excess was detected in the F_1 hybrids (one-tailed *t*-test: $P < 0.001$), as previously reported by Leary et al. (1983, 1993), as well as in the post-F_1 hybrids ($P = 0.024$).

Direction of Hybridization

Mitochondrial DNA haplotypes characteristic of the parental species indicate whether bull or brook trout females produced the F_1 hybrids (Table 3). All F_1 hybrids collected from Lion Creek ($N = 12$), One Horse Creek ($N = 9$), and Slate Creek ($N = 5$) had bull trout mtDNA haplotypes, indicating that they were produced from bull trout females and brook trout males. The F_1 hybrids collected from Mission Creek had either bull trout mtDNA (22) or brook trout mtDNA (19) haplotypes, indicating that both bull and brook trout females produced hybrids in this creek. The other 10 F_1 hybrids from Mission Creek were not analyzed with respect to mtDNA haplotype because they were discarded before DNA was extracted. The proportion of bull and brook trout mtDNA haplotypes among F_1 hybrids in the Mission Creek samples is not statistically different from 1:1 ($P = 0.757$). This result should not be interpreted, however, as indicating that equal proportions of female bull and brook trout participate in hybrid matings because the population density of brook trout is markedly higher than that of bull trout (Hansen and DosSantos 1997).

Discussion

Genetic Markers for Hybrid Detection

The accuracy of fish genetic identification depends primarily on the number of diagnostic loci rather than on the technique used because the power per allozyme or PINE locus is about the same as long as one uses the PINE fragments characteristic of only the nonnative taxon (Kanda et al. 2002). The overall probability of misidentifying a fish was thus the smallest with a combination of allozymes from muscle and liver tissue and PINEs, as this provided the greatest number of diagnostic loci.

A drawback of using allozymes for hybrid detection is that lethal sampling is usually required to examine a large number of markers (but see Van Doornik et al. 1999). This feature is generally not acceptable when threatened species like bull trout are studied. Analysis of PINEs allows one to conduct hybridization studies without sacrificing fish because DNA can be extracted from a tiny piece of fresh or frozen tissue, fins, or scales. Another advantage of PINEs is that they often reveal several independent diagnostic loci between different salmonid species from one PCR amplification (Spruell et al. 2001; Kanda et al. 2002). Other DNA markers usually target only one diagnostic locus per amplification, thereby increasing the amount of work required to screen multiple loci. Although amplification of multiple loci has become possible for microsatellites, the latter may be less suitable for hybrid detection than PINEs because their fast mutation rate results in a large amount of intraspecific variability. Thus, alleles at microsatellite loci may not be species specific for all or most populations.

PINEs are not without their weaknesses. Because of their dominant pattern of inheritance, not all individual genotypes can be determined simply. Thus, for instance, it is not possible to address the possibility that gametic phase disequilibrium exists between pairs of PINE loci in samples that appear to have come from hybrid swarms. This is important information if one wants to assess the possibility that parental individuals still exist in such populations.

Introgressive Hybridization between Bull Trout and Brook Trout

About one-quarter of the hybrids detected in this study were post-F_1. The majority of these were backcrosses to one of the parental species, but some were produced from crosses between hybrids (i.e., F_2s). There is a small possibility that some backcrosses or F_2 hybrids in our samples were misidentified as F_1 or parental fish. Thus, there may have been more post-F_1 hybrids sampled than the data indicated. Despite this, the results indicate

that F_1 hybrids between bull and brook trout are capable of reproducing in the wild more frequently than previously thought.

Our results, however, still indicate the existence of some mechanism(s) that prevent(s) these two species from forming hybrid swarms. One possibility is that F_1 and post-F_1 hybrids have reduced fitness. Reduced fitness could result from reduced fertility because of interspecific differences in chromosome numbers. The diploid number of chromosomes is 78 for bull trout and 84 for brook trout (Cavender 1984; Hartley 1987). The difference in chromosome number between the parental species may cause unmatched pairing of chromosomes during meiosis and unequal segregation, resulting in the infertility or reduced fertility of F_1 hybrids even though they may be as viable as their parents because they carry a set of chromosomes from both. This potential disruption of meiosis may be further exacerbated in post-F_1 hybrids because they do not have a set of chromosomes from both parental species.

In spite of the fact that interspecific hybridization in the genus *Salvelinus* is not a rare event (Hammar et al. 1991; Verspoor and Hammar 1991; Wilson and Hebert 1993; Baxter et al. 1997), the number of post-F_1 hybrids reported is usually low. Most reports of natural hybridization in *Salvelinus* involve pairs of species with different numbers of chromosomes: arctic char *S. alpinus* (2n = 80), brook trout (2n = 84), bull trout (2n = 78), Dolly Varden *S. malma* (2n = 82), and lake trout *S. namaycush* (2n = 84). Fertile hybrids between brook and lake trout (splake), however, have been produced under hatchery conditions for several generations, and later-generation splake are as viable as the parental species (Berst et al. 1980). The unusual fertility and viability of splake may be a consequence of the fact that lake trout and brook trout have the same chromosome number (2n = 84) and are thought to have a common but distant ancestor in eastern North America (Behnke 1972).

The observation that most F_1 hybrids between bull and brook trout are males also suggests that they have reduced fertility. It is not unusual to observe an unequal sex ratio in F_1 hybrids of fish (Hubbs 1955; Fritz and Garside 1974; Dawley et al. 1985; Dawley 1987; Mair et al. 1991). Such a ratio usually involves a preponderance of sterile males. For example, in *Lepomis* species, hybridization usually does not go beyond F_1 (Avise and Saunders 1984; Konkle and Philipp 1992) because the majority of such hybrids from both natural and artificial crosses are sterile males (Hubbs 1955; Dawley et al. 1985). In *Lepomis* species, introgression is further prevented because backcrosses between the rare fertile female hybrids and parental males produce sterile triploid fish due to an unusual meiotic mechanism (Dawley et al. 1985).

Sex determination in fishes is very plastic compared with that in other taxa (Purdom 1993). Fishes possess a wide variety of sex determination mechanisms (Kirpichnikov 1981), and autosomal or environmental factors sometimes have a stronger influence on sex determination than sex chromosomes. Sex determination in F_1 hybrids between bull and brook trout could therefore be due to a complex interaction of multiple factors.

The general rarity of female F_1 hybrids explains the rarity of F_2 or later-generation hybrids. Both sexes must obviously be present in the F_1 generation to produce F_2 progeny. Both sexes were detected only among the F_1s in Mission Creek, and not surprisingly, this was the only location in which F_2 hybrids were detected.

Another possible explanation for reduced hybrid fitness is strong selection against post-F_1 hybrids. Although limited by small sample sizes (30 F_1 hybrids and 18 post-F_1 hybrids), the length distribution of the samples from Mission Creek suggests that post-F_1 hybrids have lower survival than F_1 hybrids (Kanda 1998). Because the bull and brook trout in this creek are migratory, hybrids can grow to 700 mm, which leads to a wide range of possible sizes. While our F_1 hybrids ranged from less than 200 mm to more than 600 mm, our post-F_1 hybrids were predominantly (15 of the 18) smaller than 200 mm, suggesting that post-F_1 hybrids have reduced survival and may not attain sexual maturity.

Finally, the lack of evidence for extensive introgression between bull and brook trout could simply reflect the fact that introgression is a recent event. It will take 5 to 10 years, depending on the generation time of F_1 hybrids, for post-F_1 hybrids to appear in populations after the initial hybridization events. For instance, most of the post-F_1 hybrids from Mission Creek were collected in 1996. The population survey in that creek in 1994 found a number of adult F_1 hybrids and subsequently an unusually high number of redds. Therefore, the appearance of a considerable number of young backcross fish in Mission Creek in 1996 could be the result of introgressive hybridization that first occurred in 1994. This, however, seems unlikely because brook trout have been present in Mission Creek since at least 1973 (Hansen and DosSantos 1997). Since brook trout were first introduced into streams in Montana over 100 years

ago (Evermann 1901; MacCrimmon and Campbell 1969), it is also unlikely that all five of the populations we studied are at an initial stage of introgression.

Direction of Hybridization

Although constrained by small sample sizes, we observed unidirectional hybridization in Slate, One Horse, and Lion creeks, where all of the F_1 hybrids collected were produced from bull trout females. We have also found a location in Oregon where the majority of F_1 hybrids (61 out the 67) were produced from bull trout females (Kanda et al., unpublished data). These results indicate that the reproductive effort of bull trout that is wasted in the production of hybrids is often much greater than that based solely on the proportion of estimated hybrids in the population, since most hybrids are produced from females.

Differences in spawning time, age at maturity, abundance, and life history may affect the direction of hybridization between bull and brook trout (Hubbs 1955; Avise and Saunders 1984; Konkle and Philipp 1992; McGowan and Davidson 1992; Wilson and Hebert 1993; Kitano et al. 1994). Because brook trout males are ready to spawn earlier than females and the spawning season of brook trout is slightly later than that of bull trout, brook trout males have a better chance of mating with bull trout females than with brook trout females (Blanchfield and Ridgway 1997; Hansen and DosSantos 1997). Brook trout males also tend to mature at an earlier age than brook trout females, further increasing the chances of their being involved in hybrid matings. Hybridization also tends to occur between males from abundant species and females from rare species (Avise and Saunders 1984). Brook trout abundance is often higher than that of bull trout where hybridization has been reported (Leary et al. 1993; Buktenica 1997; Fredenberg 1997; Hansen and DosSantos 1997). Finally, small resident brook trout males can successfully spawn with large migratory bull trout females by ''sneaking'' when migratory and resident types coexist in the same stream. Assortative mating normally occurs in these situations because fish of widely different sizes seldom pair, preventing hybridization between resident female brook trout and migratory male bull trout (Foote and Larkin 1988; Gross 1991; Foote et al. 1997; James and Sexauer 1997). Kitano et al. (1994; see also Fredenberg 1997) observed that a small male brook trout released sperm after sneaking into a redd made by a pair of large bull trout in Squeezer Creek in the Swan River drainage, where large migratory bull trout and small resident brook trout coexist. This factor is probably why only hybrids between male brook trout and female bull trout were detected in Goat and Lion creeks.

We observed reciprocal crosses between bull and brook trout in Mission Creek but not in Slate and One Horse creeks, even though each creek contains individuals of both species with the same life history. In Mission Creek, both male and female bull trout may pair with brook trout more often than usual because of limited spawning habitat and the extremely low abundance of bull trout. Spawning is limited to an approximately 1-km stretch upstream from Mission Reservoir, and the number of reproductive bull trout in Mission Creek is extremely low compared with that of reproductive brook trout (Hansen and DosSantos 1997). Contrary to the situation in Mission Creek, hybridization apparently occurs only in a narrow contact zone in Slate and One Horse creeks (C. Clancy, Montana Department of Fish, Wildlife, and Parks, personal communication). The less disparate differences in population size in these contact zones and the possibly increased temporal differences in spawning time may largely restrict hybridization to male brook trout and female bull trout in these streams.

Implications for Bull Trout Conservation

The results of this study suggest that we should change our approach to bull trout conservation. Some current management practices selectively remove brook trout from streams to avoid the incidental killing of bull trout misidentified as hybrids (Buktenica 1997). This procedure would be adequate if hybrid fish were mainly sterile and did not attempt to reproduce. In this case, bull trout spawning effort would be wasted only in the production of F_1 hybrids. This study, however, indicates that reproduction by F_1 hybrids between these two fish species is widespread. The extent of this hybridization can be high at times. In the random sample from Mission Creek, for instance, we found that only 5% of the fish were bull trout and 8% were post-F_1 fish. Hybrids, therefore, can cause a further reduction of bull trout spawning success through backcrossing as well as through competition for mates and spawning sites. Although hybrid swarms between bull and brook trout apparently do not occur, the disappearance of post-F_1 hybrids caused by their apparently reduced survival would result in underestimation of the introgressive hybridization in bull trout pop-

ulations. This suggests that it would be wise to consider removing both brook trout and hybrids to avoid the further decline of bull trout populations through wasted reproductive effort. We propose that a combination of the PINE-PCR method using DNA samples extracted from fin clips and fish tagging will be the most effective way to accurately conduct removal of brook trout and hybrids. The only likely error is that some F_2 hybrids will be removed as backcrosses to brook trout.

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Appendix: Calculating Error Probabilities

TABLE A.1.—Equations for calculating the probabilities of misidentifying hybrids using allozymes and paired interspersed nuclear DNA elements (PINEs). Backcrosses may be misidentified as bull trout, brook trout, or F_1 hybrids and F_2 hybrids as bull trout, brook trout, F_1 hybrids, or backcrosses. Note that the probabilities using PINEs differ between backcrosses to bull trout (BBL) and brook trout (BBR) because the number of diagnostic fragments differs between the species. Symbols are defined as follows: x = the number of diagnostic allozyme loci, y = the number of bull-trout-specific PINE fragments, and z = the number of brook-trout-specific PINE fragments.

	Backcross errors			F_2 errors			
Marker	Bull trout	Brook trout	F_1 hybrids	Bull trout	Brook trout	F_1 hybrids	BBL, BBR
Allozymes	$(1/2)^x$	$(1/2)^x$	$(1/2)^x$	$(1/4)^x$	$(1/4)^x$	$(1/2)^x$	$\sum_{i=1}^{x-1} \{(1/4)^i(1/2)^{x-i}[x!/(i!x - i)]\}$
PINEs	$(1/2)^z$	$(1/2)^y$	BBL = $(1/2)^z$	$(3/4)^y(1/4)^z$	$(1/4)^y(3/4)^z$	$(3/4)^{y+z}$	BBL = $(3/4)^y \sum_{i=1}^{z-1} \{(3/4)^i(1/4)^{z-i}[z!/(i!z - i)]\}$
			BBR = $(1/2)^y$				BBR = $(3/4)^z \sum_{i=1}^{y-1} \{(3/4)^i(1/4)^{y-i}[y!/(i!y - i!)]\}$